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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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07/997,734 30 December 1992 (30.12.92) US

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(81) Designated States: JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

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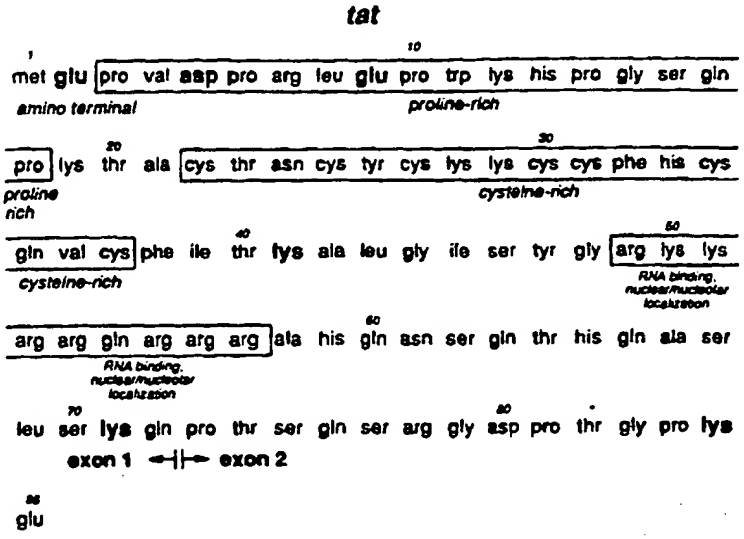
(54) Title: TAT AND REV OLIGOPEPTIDES IN HIV TREATMENT



Primary amino acid sequence of the HIV HXB2 tat protein. Acidic residues are indicated in bold face. The proline-rich and basic regions are indicated. The basic region is also the region which binds RNA and specifies nuclear/nucleolar localization (boxed).

(57) Abstract

The invention concerns peptides corresponding the Rev regulatory protein of the Human Immunodeficiency virus and methods of using the peptides.

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TAT & REV OLIGOPEPTIDES IN HIV TREATMENT

Field of the Invention

This invention relates to the therapeutic use of synthetic oligopeptides homologous to the signal sequences of viral regulatory proteins in the treatment of HIV infection.

References

1. Haseltine WA. 1991. In Genetic Structure and Regulation of HIV. Haseltine W.A., Wong-Staal F. eds. Harvard AIDS Institutes Series on Gene Regulation of Human Retroviruses Vol. 1, Raven Press New York: 1.

2. Kuppuswamy M. et al. 1989. Nucl. Acid. Res. 17: 3551.

Background

Two of the essential regulatory proteins of HIV are the transactivator protein (tat) and the regulatory virion protein (rev). The binding of these proteins to specific regions of the viral RNA are essential for RNA function and for viral replication (review in 1). Inhibition of this binding mechanism leads to new therapeutic options in the treatment of AIDS.

In this patent application the conventional three letter abbreviation for amino acids is used and the peptide sequences given are read from the amino-terminal end to the carboxyl-terminal end.

Summary of the Invention

Tat and rev belong to a group of proteins essential for the regulation of HIV expression. The signal sequences of tat protein and rev protein for their interaction with HIV RNA are oligopeptide sequences enriched in cationic amino acids. The cationic charge of these signal sequences is essential for the binding of these proteins to the transacting responsive region (TAR) of HIV RNA and for RNA function. Inhibition of these binding mechanisms between tat and rev on one side and HIV RNA on the other side inhibits viral replication. Therapeutically this inhibition can be achieved in two ways. i) The parenteral application of synthetic analogs to the signal sequences of viral regulatory proteins, particularly to their RNA binding region. In this case the therapeutic mechanism is the competitive inhibition of the binding of viral regulatory proteins to viral RNA. This therapeutic approach may be particularly valuable in the treatment of manifest HIV infections and AIDS. ii). By subcutaneous or intracutaneous injection of these synthetic analogs to the peptide signal sequences of the viral regulatory proteins. In this case the antibodies produced against these antigens would block the signal sequences of these regulatory proteins and prevent the interaction with viral RNA. In this case a vaccine will be available in the prevention and treatment of HIV infections and AIDS.

Detailed Description of the Invention

The human immunodeficiency viruses (HIV) are etiologically linked with the acquired immunodeficiency syndrome (AIDS). Initial HIV infections appear to be silent with no or low levels of viral replication. Disease progression leads to a state of prolific viral replication and cell death. The different stages of HIV replication are determined by regulatory mechanisms controlling gene expression. In addition to the genes for viral structural proteins the HIV genom includes other genes encoding for virus-associated regulatory proteins. Among these viral regulatory proteins tat and rev are of particular importance (review in 1).

Transactivator Protein (tat)

Tat is a strong transactivator of genes that are expressed from the viral long terminal repeat (LTR) and is essential for HIV gene expression, viral replication, and virus mediated cythopathicity. The tat protein is 86 to 102 amino acids long, dependent on the viral strain. The sequence of the HIV HXB2 tat protein is given in figure 1. Several highly conserved domains in the tat protein have been identified among the various strains of HIV-1, HIV-2 and simian immunodeficiency virus (SIV)(2). The following domains are essential for tat function:

1). A domain rich in proline residues as well as cationic amino acid residues (arginine, lysine, histidine) and anionic amino acid residues (glutamate, aspartate): Glu-Pro-Val-Asp-Pro-Arg-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser-Gln-Pro-Lys (residue 2 to 19, figure 1)

2). A domain containing seven cysteine residues: Cys-Thr-Asn-Cys-Tyr-Cys-Lys-Lys-Cys-Cys-Phe-His-Cys-Gln-Val-Cys (residue 22 to 37, figure 1)

3). A domain containing the motif: Lys-X-Leu-Gly-Ile-X-Tyr (residue 41 to 47, figure 1). In this motif the residue lysine is essential and the residues glycine and tyrosine are partially essential for tat function.

4). A strong basic domain Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg (residue 49 to 57, figure 1). This basic region specifies both RNA binding and nuclear/nucleolar localization.

The claims of this patent are based on the following reported findings and new discoveries:

1. The information for the entire biological activity of the tat protein are contained in specific oligopeptide sequences within the tat peptide sequence (2).

2. These oligopeptide signal sequences are highly antigenic.

3. The activity of the tat protein can be inhibited in two ways:

a) by the competitive inhibition of its binding to viral RNA via synthetic analogs of the

signal sequences

b) by antibodies raised against these signal sequences which block the binding of transactivating proteins to viral RNA

4. Because of its defined binding properties to viral RNA synthetic analogs to the strongly basic oligopeptide of viral regulatory proteins are the most promising synthetic peptides for therapeutic purposes.

The therapeutic implications of this invention are the following:

1). A parenteral solution containing synthetic analogs to one or more of the signal sequences of viral regulatory proteins. This therapeutic application would be preferentially used, but is not limited to the treatment of manifest HIV infection or AIDS.

2). The subcutaneous or intracutaneous application of synthetic analogs to one or more of the signal sequences of viral regulatory proteins. In this case the oligopeptides would be used a vaccine in the prevention and treatment of HIV infections and AIDS.

Regulatory Virion Protein (rev)

The regulator of virion protein expression (rev) is another regulatory protein involved in HIV gene expression and pathogenicity (review in 1). The tat and rev regulatory pathways share much in common. The virus life cycle is divided into an early phase during which tat and rev proteins are made and a late phase in which virus structural proteins are made and virus particles are assembled. The rev protein mediates the switch from the early to the late phase. The sequence of HIV-1 3HXB2 rev protein is given in figure 2.

The following domains are highly conserved among the rev proteins of different types of HIV viruses and they have been shown to be essential for rev function:

1. The domain: Pro-Pro-Pro-Asn-Pro (residues 27 to 31, figure 2)
2. The domain: Arg-Gln-Ala-Arg-Arg-Asn-Arg-Arg-Arg-Arg-Trp-Arg-Glu-Arg-Gln-Arg- (residues 35 to 50, figure 2).
3. The domain: Leu-Gln-Leu-Pro-Pro-Leu-Glu-Arg-Leu-Thr-Leu (residue 73 to 83, figure 2)

Analogous to the tat protein the strong basic amino acid region of the rev protein determines its binding to RNA and also specifies nuclear and nucleolar localization. This peptide region of the rev protein is responsible for the specific binding of rev to the rev responsive element (RRE), a short sequence in the gene for the viral envelope glycoprotein.

The claims of this patent and the therapeutic implications for synthetic analogs of rev protein sequences are based on inventions analogous to those described for the tat protein.

Experimental Design

The inventions disclosed in this patent application can be tested in the following way:

In vitro:

1. The inhibitory effect of synthetic peptides on transactivation can be measured *in vitro* by competition experiments measuring the decrease of expression of the bacterial chloramphenicol acetyltransferase (CAT) reporter gene linked to the HIV-LTR as described in (2) or by using another assay measuring transactivation or viral replication in the presence of these synthetic peptides.

2. The inhibitory effect of antibodies against synthetic peptides analogous to the signal sequences of viral regulatory proteins can be tested *in vitro* in the following way. Polyclonal or monoclonal antibodies are raised against specific signal peptides of viral regulatory proteins. The inhibitory effect of these antibodies on transactivation and viral replication can be measured in assays similar to those described above.

In vivo:

3. The therapeutic effect of synthetic peptides analogous to the signal sequences of viral regulatory proteins can be tested *in vivo* in suitable animal models susceptible to SIV. Therapeutic dosages of these synthetic peptides, given intravenously, decrease the susceptibility of the test animals to HIV exposure. Therapeutic dosages of these synthetic peptides given intravenously can also be tested for reducing the clinical symptoms of AIDS.

4. In an analogous way the synthetic peptides analogous to the signal sequences of viral regulatory proteins can be tested *in vivo* as vaccines in the prevention and treatment of SIV in suitable animal models. Adequate amounts of these synthetic peptides are injected subcutaneously or intracutaneously as antigens. The effect of this vaccination can be studied by assessing the development of symptoms of HIV infection and AIDS.

Claims

The following amino acid sequences and their therapeutic use are claimed:

1. INFORMATION FOR SEQ ID NO 1:
(A) LENGTH 6
(B) TYPE: amino acid
(D) TOPOLOGY: linear

Arg Arg Gln Arg Arg Arg

2. INFORMATION FOR SEQ ID NO 2:
(A) LENGTH 6
(B) TYPE: amino acid
(D) TOPOLOGY: linear

Arg Arg Asn Arg Arg Arg

3. INFORMATION FOR SEQ ID NO 3:
(A) LENGTH 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

Arg Lys Lys Arg Arg Gln Arg Arg Arg Ala

4. INFORMATION FOR SEQ ID NO 4:
(A) LENGTH 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

Arg Gln Ala Arg Arg Asn Arg Arg Arg Arg

5. INFORMATION FOR SEQ ID NO 5:
(A) LENGTH 17
(B) TYPE: amino acid
(D) TOPOLOGY: linear

Arg Gln Ala Arg Arg Asn Arg Arg Arg Arg Trp Arg Glu Arg Gln Arg Gln

6. INFORMATION FOR SEQ ID NO 6:
(A) LENGTH 24
(B) TYPE: amino acid
(D) TOPOLOGY: linear

Arg Lys Lys Arg Arg Gln Arg Arg Arg Ala His Gln Asn Ser Gln Thr His Gln Ala Ser Leu Ser Lys Gln

7. INFORMATION FOR SEQ ID NO 7:
(A) LENGTH 18
(B) TYPE: amino acid
(D) TOPOLOGY: linear

Glu Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser Gln Pro Lys

8. INFORMATION FOR SEQ ID NO 8:
(A) LENGTH 16
(B) TYPE: amino acid
(D) TOPOLOGY: linear

Cys Thr Asn Cys Tyr Cys Lys Lys Cys Cys Phe His Cys Gln Val Cys

9 INFORMATION FOR SEQ ID NO 9:
(A) LENGTH 24
(B) TYPE: amino acid
(D) TOPOLOGY: linear

10. INFORMATION FOR SEQ ID NO 10:
(A) LENGTH 7
(B) TYPE: amino acid
(D) TOPOLOGY: linear

Lys Ala Leu Gly Ile Ser Tyr

11. INFORMATION FOR SEQ ID NO 11:
(A) LENGTH 6
(B) TYPE: amino acid
(D) TOPOLOGY: linear

Pro Pro Pro Asn Pro Glu

12. INFORMATION FOR SEQ ID NO 12:
(A) LENGTH 13
(B) TYPE: amino acid
(D) TOPOLOGY: linear

Pro Leu Gln Leu Pro Pro Leu Glu Arg Leu Thr Leu Asp

13. Peptide sequences analogous to the signal sequences of viral regulatory proteins according to claims 1 to 12 in which one or more amino acid residues are substituted with amino acid residues of identical polarity and charge.

14. Peptide sequences analogous to the signal sequences of viral regulatory proteins according to claims 1 to 13 in which one or more amino acid residues are added and/or deleted from the amino terminal and/or carboxyl-terminal end of the represented sequence.

15. The therapeutic use of one or more synthetic peptides analogous to the signal sequences of viral regulatory proteins according to claim 1 to 14 in the treatment of HIV infection comprising a pharmaceutically acceptable carrier for parenteral administration.

16. The therapeutic parenteral use of one or more synthetic peptides analogous to the signal sequences of viral regulatory proteins according to claim 1 to 15 as a competitive inhibitor for the activation of tat and rev proteins and thereby for the function of viral RNA in the treatment of HIV infection.

17. A composition according to Claims 1 to 16 further comprising one or more of the following compounds: ascorbate, N-acetylcysteine, glutathione and/or suitable salts thereof.

18. The therapeutic use of one or more synthetic peptides analogous to the signal sequences of viral regulatory proteins according to claim 1 to 14 comprising a pharmaceutically acceptable carrier for subcutaneous and/or intracutaneous administration.

19. The therapeutic use of one or more synthetic peptides analogous to the signal sequences of viral regulatory proteins according to claim 1 to 14 as a vaccine in the prevention and treatment of HIV infection.

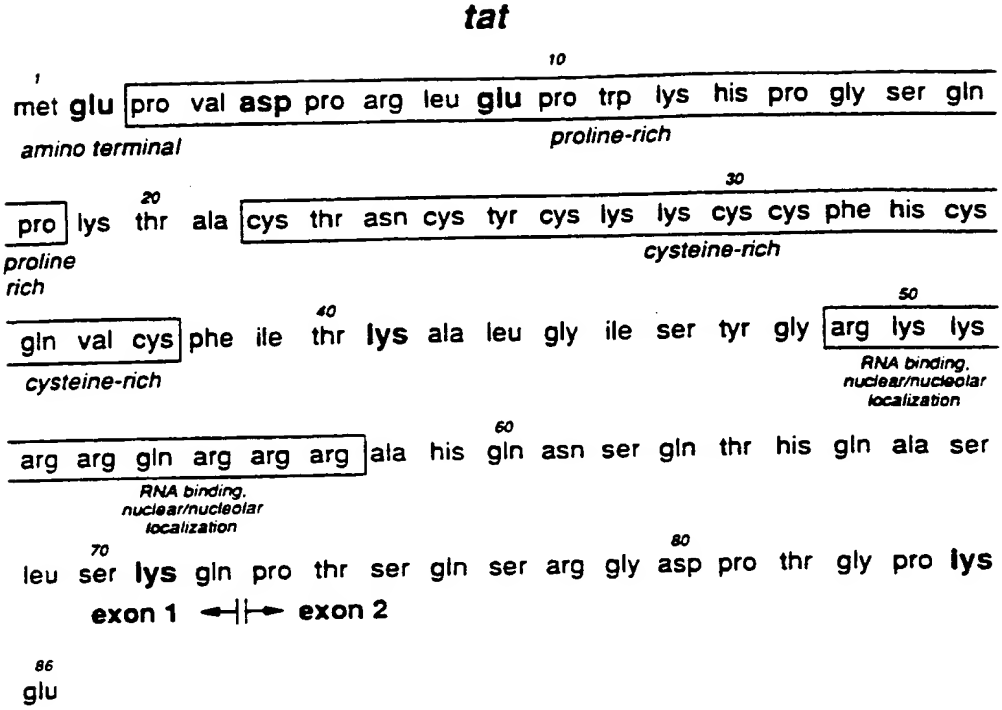

Figure 1. Primary amino acid sequence of the HIV HXB2 tat protein. Acidic residues are indicated in bold face. The proline-rich and basic regions are indicated. The basic region is also the region which binds RNA and specifies nuclear/nucleolar localization (boxed).

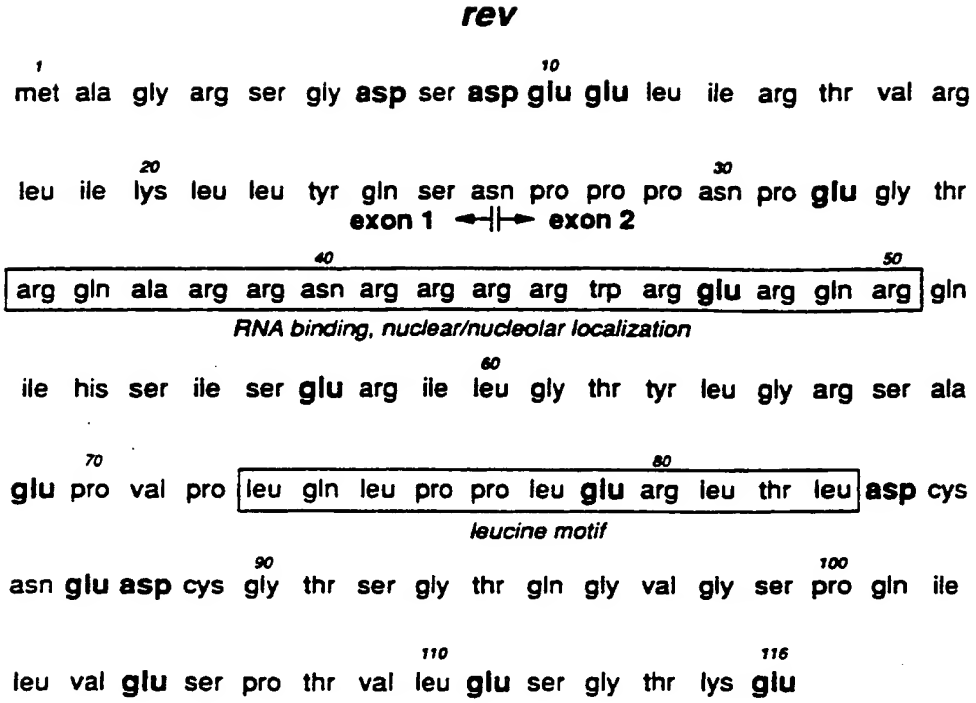

Figure 2: Primary amino acid sequence of the HIV-1 3HXB2 rev protein. The basic regions, which specifies both RNA binding and nuclear/nucleolar localization, as well as the leucine-rich region are blocked. Acidic residues are indicated in bold face.

INTERNATIONAL SEARCH REPORT

Ir. .ational application No.
PCT/US93/12680

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 39/12, 37/02; C07K 5/00, 15/00, 7/00, 17/00
US CL : 424/89; 530/328, 350, 329
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/89; 530/328, 350, 329

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog, Search terms: HIV, tat, rev, oligopeptide, treatment

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	CELL, VOLUME 58, ISSUED 14 JULY 1989, MALIM ET AL., "FUNCTIONAL DISSECTINOF THE HIV-1 REV TRNS-ACTIVATIOR-DERIVATIONOF A TRANS-DOMINANT REPRESSOR OF REV FUNCTION", PAGES 205-214, ESPECIALLY PAGES 205-206.	1-14 -------- 15-18
Y	THE EMBO JOURNAL, VOLUME 11, NO. 3, ISSUED 1992, KJEMS ET AL., "SPECIFIC BINDING OF A BASIC PEPTIDE FROM HIV-1 REV", PAGES 1119-1129, ESPECIALLY PAGE 1128.	1-14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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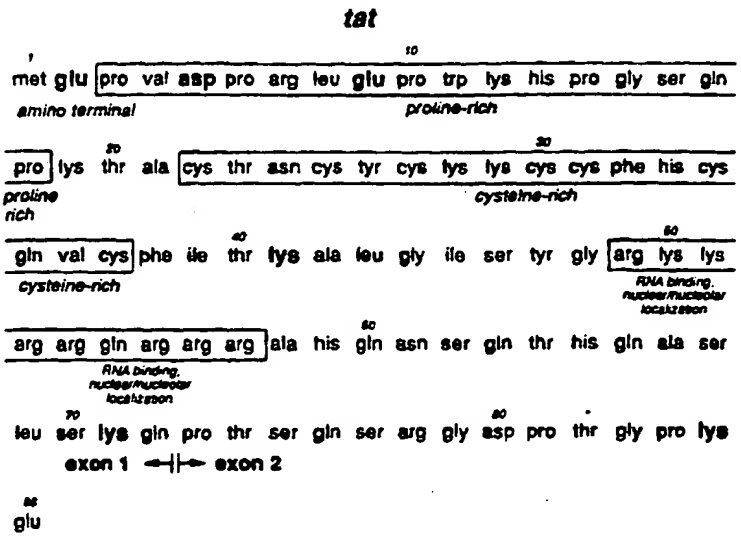

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4. Because of its defined binding properties to viral RNA synthetic analogs to the strongly basic oligopeptide of viral regulatory proteins are the most promising synthetic peptides for therapeutic purposes.

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The following domains are highly conserved among the rev proteins of different types of HIV viruses and they have been shown to be essential for rev function:

1. The domain: Pro-Pro-Pro-Asn-Pro (residues 27 to 31, figure 2)
2. The domain: Arg-Gln-Ala-Arg-Arg-Asn-Arg-Arg-Arg-Arg-Trp-Arg-Glu-Arg-Gln-Arg- (residues 35 to 50, figure 2).
3. The domain: Leu-Gln-Leu-Pro-Pro-Leu-Glu-Arg-Leu-Thr-Leu (residue 73 to 83, figure 2)

Analogous to the tat protein the strong basic amino acid region of the rev protein determines its binding to RNA and also specifies nuclear and nucleolar localization. This peptide region of the rev protein is responsible for the specific binding of rev to the rev responsive element (RRE), a short sequence in the gene for the viral envelope glycoprotein.

The claims of this patent and the therapeutic implications for synthetic analogs of rev protein sequences are based on inventions analogous to those described for the tat protein.

Experimental Design

The inventions disclosed in this patent application can be tested in the following way:

In vitro:

1. The inhibitory effect of synthetic peptides on transactivation can be measured *in vitro* by competition experiments measuring the decrease of expression of the bacterial chloramphenicol acetyltransferase (CAT) reporter gene linked to the HIV-LTR as described in (2) or by using another assay measuring transactivation or viral replication in the presence of these synthetic peptides.

2. The inhibitory effect of antibodies against synthetic peptides analogous to the signal sequences of viral regulatory proteins can be tested *in vitro* in the following way. Polyclonal or monoclonal antibodies are raised against specific signal peptides of viral regulatory proteins. The inhibitory effect of these antibodies on transactivation and viral replication can be measured in assays similar to those described above.

In vivo:

3. The therapeutic effect of synthetic peptides analogous to the signal sequences of viral regulatory proteins can be tested *in vivo* in suitable animal models susceptible to SIV. Therapeutic dosages of these synthetic peptides, given intravenously, decrease the susceptibility of the test animals to HIV exposure. Therapeutic dosages of these synthetic peptides given intravenously can also be tested for reducing the clinical symptoms of AIDS.

4. In an analogous way the synthetic peptides analogous to the signal sequences of viral regulatory proteins can be tested *in vivo* as vaccines in the prevention and treatment of SIV in suitable animal models. Adequate amounts of these synthetic peptides are injected subcutaneously or intracutaneously as antigens. The effect of this vaccination can be studied by assessing the development of symptoms of HIV infection and AIDS.

Claims

The following amino acid sequences and their therapeutic use are claimed:

1. INFORMATION FOR SEQ ID NO 1:
(A) LENGTH 6
(B) TYPE: amino acid
(D) TOPOLOGY: linear

Arg Arg Gln Arg Arg Arg

2. INFORMATION FOR SEQ ID NO 2:
(A) LENGTH 6
(B) TYPE: amino acid
(D) TOPOLOGY: linear

Arg Arg Asn Arg Arg Arg

3. INFORMATION FOR SEQ ID NO 3:
(A) LENGTH 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

Arg Lys Lys Arg Arg Gln Arg Arg Arg Ala

4. INFORMATION FOR SEQ ID NO 4:
(A) LENGTH 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

Arg Gln Ala Arg Arg Asn Arg Arg Arg Arg

5. INFORMATION FOR SEQ ID NO 5:
(A) LENGTH 17
(B) TYPE: amino acid
(D) TOPOLOGY: linear

Arg Gln Ala Arg Arg Asn Arg Arg Arg Arg Trp Arg Glu Arg Gln Arg Gln

6. INFORMATION FOR SEQ ID NO 6:
(A) LENGTH 24
(B) TYPE: amino acid
(D) TOPOLOGY: linear

Arg Lys Lys Arg Arg Gln Arg Arg Arg Ala His Gln Asn Ser Gln Thr His Gln Ala Ser Leu Ser Lys Gln

7. INFORMATION FOR SEQ ID NO 7:
(A) LENGTH 18
(B) TYPE: amino acid
(D) TOPOLOGY: linear

Glu Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser Gln Pro Lys

8. INFORMATION FOR SEQ ID NO 8:
(A) LENGTH 16
(B) TYPE: amino acid
(D) TOPOLOGY: linear

Cys Thr Asn Cys Tyr Cys Lys Lys Cys Cys Phe His Cys Gln Val Cys

9 INFORMATION FOR SEQ ID NO 9:
(A) LENGTH 24
(B) TYPE: amino acid
(D) TOPOLOGY: linear

10. INFORMATION FOR SEQ ID NO 10:
(A) LENGTH 7
(B) TYPE: amino acid
(D) TOPOLOGY: linear

Lys Ala Leu Gly Ile Ser Tyr

11. INFORMATION FOR SEQ ID NO 11:
(A) LENGTH 6
(B) TYPE: amino acid
(D) TOPOLOGY: linear

Pro Pro Pro Asn Pro Glu

12. INFORMATION FOR SEQ ID NO 12:
(A) LENGTH 13
(B) TYPE: amino acid
(D) TOPOLOGY: linear

Pro Leu Gln Leu Pro Pro Leu Glu Arg Leu Thr Leu Asp

13. Peptide sequences analogous to the signal sequences of viral regulatory proteins according to claims 1 to 12 in which one or more amino acid residues are substituted with amino acid residues of identical polarity and charge.

14. Peptide sequences analogous to the signal sequences of viral regulatory proteins according to claims 1 to 13 in which one or more amino acid residues are added and/or deleted from the amino terminal and/or carboxyl-terminal end of the represented sequence.

15. The therapeutic use of one or more synthetic peptides analogous to the signal sequences of viral regulatory proteins according to claim 1 to 14 in the treatment of HIV infection comprising a pharmaceutically acceptable carrier for parenteral administration.

16. The therapeutic parenteral use of one or more synthetic peptides analogous to the signal sequences of viral regulatory proteins according to claim 1 to 15 as a competitive inhibitor for the activation of tat and rev proteins and thereby for the function of viral RNA in the treatment of HIV infection.

17. A composition according to Claims 1 to 16 further comprising one or more of the following compounds: ascorbate, N-acetylcysteine, glutathione and/or suitable salts thereof.

18. The therapeutic use of one or more synthetic peptides analogous to the signal sequences of viral regulatory proteins according to claim 1 to 14 comprising a pharmaceutically acceptable carrier for subcutaneous and/or intracutaneous administration.

19. The therapeutic use of one or more synthetic peptides analogous to the signal sequences of viral regulatory proteins according to claim 1 to 14 as a vaccine in the prevention and treatment of HIV infection.

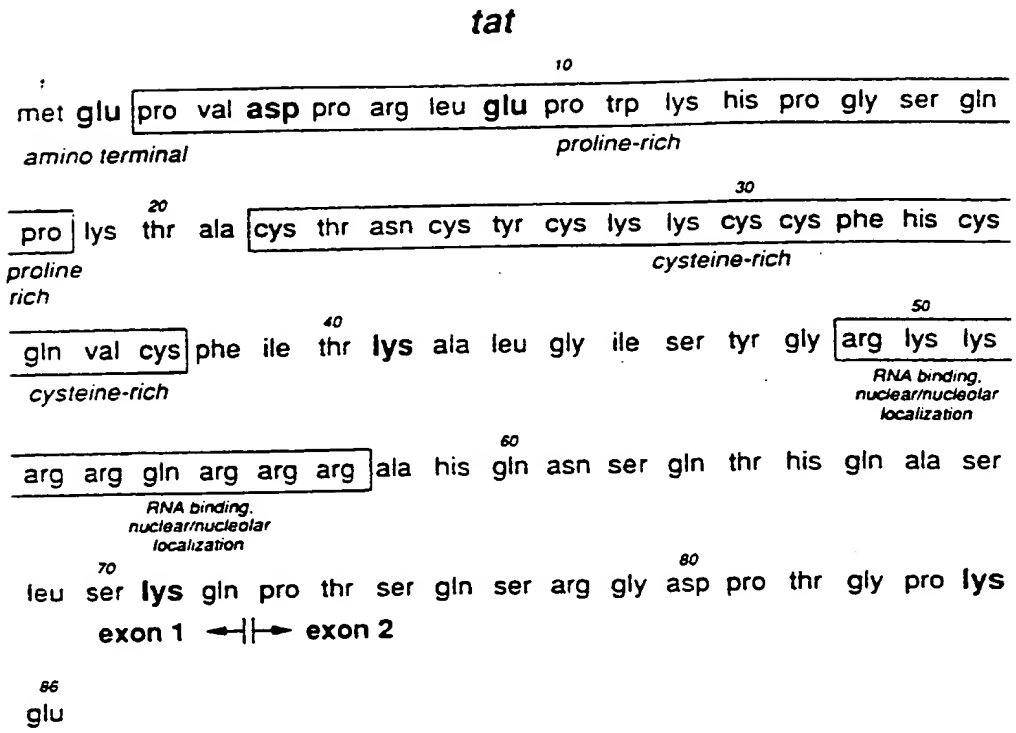

Figure 1. Primary amino acid sequence of the HIV HXB2 tat protein.

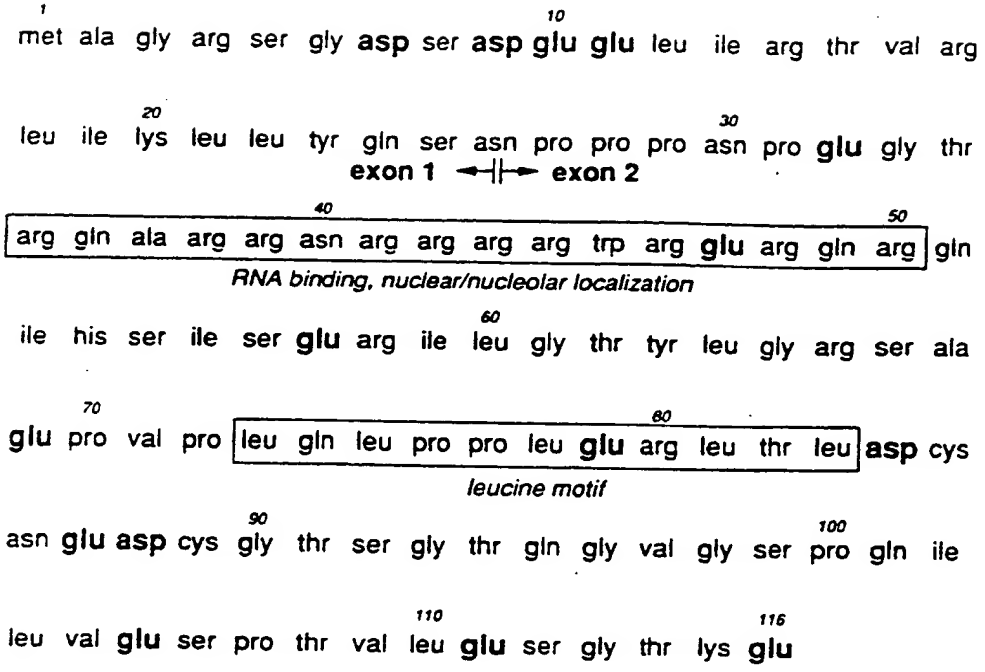

Figure 2: Primary amino acid sequence of the HIV-1 3HXB2 rev protein.

INTERNATIONAL SEARCH REPORT

Ir. .ational application No.
PCT/US93/12680

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 39/12, 37/02; C07K 5/00, 15/00, 7/00, 17/00
US CL : 424/89; 530/328, 350, 329
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/89; 530/328, 350, 329

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog, Search terms: HIV, tat, rev, oligopeptide, treatment

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	CELL, VOLUME 58, ISSUED 14 JULY 1989, MALIM ET AL., "FUNCTIONAL DISSECTINOF THE HIV-1 REV TRNS-ACTIVATIOR-DERIVATIONOF A TRANS-DOMINANT REPRESSOR OF REV FUNCTION", PAGES 205-214, ESPECIALLY PAGES 205-206.	1-14 -------- 15-18
Y	THE EMBO JOURNAL, VOLUME 11, NO. 3, ISSUED 1992, KJEMS ET AL., "SPECIFIC BINDING OF A BASIC PEPTIDE FROM HIV-1 REV", PAGES 1119-1129, ESPECIALLY PAGE 1128.	1-14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 14 APRIL 1994	Date of mailing of the international search report MAY 13 1994
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